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EFFECTS OF SIMULATED SPACE ENVIRONMENTS ON THE VIABILITY OF MICROORGANISMS

Final Report
April 15, 1961 through April 30, 1963

Gerald J. Silverman, (MIT)
Rosario P. Giammanco, ~~(NRC)~~
Norman S. Davis, (MIT)
Frank C. Benner, and ~~(NRC)~~
Cecil G. Dunn (MIT)

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Approved: Norman Beecher
Norman Beecher
Assistant Director
of Research

Reviewed: John V. E. Hansen
John V. E. Hansen
Director of Marketing
and Contracts

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INTRODUCTION

This report constitutes the final summary report covering research conducted under Contract NASr-41, "Effects of Simulated Space Environment on the Viability of Microorganisms", for the National Aeronautics and Space Administration, Washington, D. C., during the period April 15, 1961 to April 30, 1963.

The objective of this project was to determine the capability of selected microorganisms to survive a combination of ultrahigh vacuum and certain stress conditions which may be encountered in outer space.

The experimental program was a joint effort of National Research Corporation through its Space Vacuum Laboratory - Research Division, and the Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

During the course of the program, selected spores and soil organisms were studied under the combined environments of

A) Ultrahigh vacuum (10^{-8} to 10^{-10} torr), and temperature (-190°C to +170°C).

B) Ultrahigh vacuum and gamma radiation.

Viability under these conditions was compared to control samples.

This work has been reported in two publications to which the readers of this report are referred for details. The titles of the publications and their abstracts follow. Copies of the papers are included in the Appendix.

COMBINED EFFECTS OF ULTRAHIGH VACUUM AND TEMPERATURE ON THE VIABILITY OF SOME SPORES AND SOIL ORGANISMS.

Authors: Norman S. Davis, Gerald J. Silverman, and Wayne H. Keller. Department of Nutrition and Food Science, Massachusetts Institute of Technology, and Research Division, National Research Corporation, Cambridge, Massachusetts.

Applied Microbiology, Vol. 11, No. 3, May 1963.

NEXT

ABSTRACT

Considerably fewer spores of Bacillus stearothermophilus, Bacillus megaterium, and Clostridium sporogenes were recovered than were spores of Bacillus subtilis var. niger and Aspergillus niger after 4 to 5 days at 53°C and 60°C in ultrahigh vacuum. There were no significant differences in the recoveries of these five organisms at 25°C and atmospheric pressure, and after exposure to 25°C and -190°C in vacuum. At 60°C, a far greater decrease in viability was demonstrated for B. stearothermophilus, B. megaterium, and C. sporogenes in ultrahigh vacuum than at atmospheric pressure. Viable B. subtilis var. niger spores were not detected in an initial 10^7 spores after retention at 90°C and ultrahigh vacuum, and 10^4 spores were viable after 5 days at 90°C and atmospheric pressure from an initial 10^6 spores. Molds and actinomycetes in soil were particularly resistant up to 69°C in vacuum. Actinomycetes were the only soil organisms recovered so far at 120°C.

Four groups of organisms in soil, mesophilic aerobes and anaerobes, molds and actinomycetes were similarly tested in the vacuum chamber. From one soil sample only actinomycetes survived 120°C, whereas one other soil sample yielded viable bacteria after exposures to 170°C. The resistance of the isolates to high temperatures in the absence of soil is being evaluated. Several organisms have been found to resist 120°C in ultrahigh vacuum for 4 to 5 days, and experiments at higher temperatures are in progress.

When irradiated with gamma rays from a Cobalt-60 source, there was a difference between vacuum dried test spores irradiated while still under vacuum to those exposed to air immediately before irradiation. A reduction of from one-third to one-ninth of the viability of spores irradiated in vacuum occurred with vacuum-treated spores irradiated in air.

OTHER WORK

In addition to the work reported in these papers, a series of preliminary experiments was conducted on the combined effects of ultraviolet radiation and ultrahigh vacuum.

Ultraviolet radiation was provided by means of a GE 15T8 germicidal lamp mounted centrally in a 20 inch diameter stainless steel framework located inside the ultrahigh vacuum system. Filters used as supports for the microorganisms were located 10 cm from the central lamp source. The lamp was

EXPOSURE OF MICROORGANISMS TO SIMULATED EXTRATERRESTRIAL SPACE ECOLOGY.

Authors: Norman S. Davis, Gerald J. Silverman and Wayne H. Keller. Department of Nutrition and Food Science, Massachusetts Institute of Technology, and Research Division, National Research Corporation, Cambridge, Massachusetts. Presented at COSPAR Symposium on Terrestrial Life in Space, Warsaw, Poland, June 1963.

ABSTRACT ✓ 15191

Spores of five test organisms, Bacillus subtilis var. niger, Bacillus megaterium, Bacillus Stearothermophilus, Clostridium sporogenes and Aspergillus niger and soils were exposed, while under ultrahigh vacuum to temperatures of from -190°C to +170°C for 4 - 5 days.

Up to a temperature of 25°C, no loss in viability of the test spores was noted when compared to original populations maintained at room temperature at atmospheric pressure over a desiccant. At elevated temperatures, differences in resistivity occurred so that at 88°C only B. subtilis var. niger and A. niger survived in appreciable numbers. At 107°C only A. niger spores survived, but none were recoverable after exposure to 120°C. In comparison, B. subtilis var. niger survived at atmospheric pressure and 90°C for 5 days while none of the other spores were viable after 24 hours.

Author

energized for short periods of time after the test specimens had been exposed for five days to ultrahigh vacuum. A 30-second exposure corresponded to 1×10^5 ergs/cm^2, a ten-minute exposure to 2×10^6 ergs/cm^2.

Spores included in the study were Bacillus stearothermophilus, B. megaterium, B. subtilis var. niger, Clostridium sporogenes and Aspergillus niger.

When membrane filters holding 10^6 spores were given 1×10^5 ergs/cm^2 at UV in vacuum and at atmospheric pressure, 0.01 - 0.1% of the spores were recovered. Differences in survival between samples exposed in vacuum to those exposed in air were observed, but these differences were not significant because of the small number of surviving spores. For spores supported on glass filters, survival ranged between 20% and 50%, except for B. subtilis var. niger, for which only 0.1% viability was noted.

At 2×10^6 ergs/cm^2, about 0.001% of the spores or membrane filters were recovered, with the exception of Aspergillus niger which gave about 0.10% survival. On glass filters, 0.01% of the B. subtilis var. niger spores, 0.5% of the A. niger spores and 10% of the other three spores were viable, based on comparison to specimens exposed to vacuum and shielded from UV.

It is planned in future work to provide means for obtaining lower UV irradiation under controlled conditions.

APPENDIX

EXPOSURE OF MICROORGANISMS TO SIMULATED EXTRATERRESTRIAL SPACE ECOLOGY

by

Gerald S. Silverman and Norman S. Davis (MIT)

and

Wayne H. Keller (NRC)

Presented at the Cospar Sixth Plenary Meeting and
Fourth International Space Science Symposium
3 - 12 June 1963
Warsaw, Poland

ACKNOWLEDGEMENT

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EXPOSURE OF MICROORGANISMS TO SIMULATED EXTRATERRESTRIAL SPACE ECOLOGY

In order to survive exposure to the stresses of outer space environments a microorganism either must possess an inherent resistant capability or be protected. Certain of these stress parameters have been simulated in ground test systems. It is the intention of the authors to describe certain of the experimental requirements necessary for adapting ultrahigh vacuum equipment to microbiological studies as well as certain results obtained from these studies conducted over the last year and one-half. This investigation was conducted by National Research Corporation, Cambridge, Massachusetts, in conjunction with MIT.

A number of studies have examined the effects of high and ultrahigh vacuum on microorganisms at ambient temperatures. Prince and Bakanauskas (1958) and Prince (1960) noted that freeze-dried spores of Aspergillus niger, A. flavus, Bacillus globigii and B. mycoides survived up to 32 days in vacuums ranging from 1×10^{-5} to 5×10^{-7} torr.

Portner et al. (1961) exposed B. subtilis, A. niger, A. Terreus and Penicillium citrinum to vacuum in the ultrahigh range (3.6×10^{-10} torr) at ambient temperature for 5 days and concluded that these organisms would survive the vacuum of outer space. Brueschke, Suess, and Willard (1961) subjected spores of B. subtilis, A. niger, A. terreus and P. citrinum to vacuums of 1×10^{-6} to 6×10^{-9} torr. P. citrinum was not recovered, but the other test organisms were recovered after 10 days at which time a vacuum of 8×10^{-8} torr was achieved. None of the organisms were viable after 30 days, at 1.2×10^{-8} torr. Subsequent publications by Morelli, Fehlner, and Stembridge (1962a, b) demonstrated, in agreement with Prince and Portner et al., that B. subtilis var. niger spores survived 35 days at 10^{-8} torr at room temperature with little or no destruction.

EXPOSURE OF MICROORGANISMS TO SIMULATED EXTRATERRESTRIAL SPACE ECOLOGY

This investigation is concerned with the viability of spores of 5 test organisms and those present in soils after exposure to a wide range of temperatures, or to gamma radiation, while under ultrahigh vacuum. The vacuums employed were from 10^{-8} to 10^{-10} torr, corresponding to an altitude of from approximately 200 to 400 miles.

The microbial assay methods and procedures employed in this study have been described elsewhere (Davis, Silverman, and Keller, 1963) and will not be discussed in detail. Spores of B. megaterium, B. subtilis var. niger, B. stearothermophilus, Clostridium sporogenes and A. niger were the test organisms. These organisms were, after suitable heat-activation where necessary, impinged upon glass fiber filter paper, dried at 45°C for 2 1/2 hours and maintained in a desiccator over silica gel until they were inserted in the vacuum apparatus. The duration of an experiment was 5 days, this period including the time required for final temperature and vacuum attainment.

One local soil and one obtained from the Mohave desert were also subjected to temperature-vacuum experiments. The more moist local soil was predried overnight at 37°C and sieved through a 48 mesh screen. In order to prevent excessive pressures in the vacuum chamber when the system was heated, the amount of soil for any experiment was limited to 5 1-g portions which were distributed in petri dishes. The control and vacuum chamber exposed soils were assayed for heterotrophic mesophilic aerobes, anaerobes, actinomycetes, molds, and in certain experiments thermophiles.

Vacuum-Temperature Equipment

For most of the experiments at elevated temperatures the apparatus described in Fig. 1 was used. By careful placement of the resistance heaters a temperature gradient within the chamber was obtained, each shelf being subjected to a different temperature zone as illustrated in Fig. 2. It is also seen that vacuum is altered by the extent of outgassing from the metal surfaces. The higher the final chamber temperature the longer the delay in achieving the ultrahigh vacuum range. In subsequent experiments employing temperatures in excess of 120°C, smaller chambers were used and the sample was subjected to a single temperature. By cooling the chamber walls, so as to minimize outgassing, vacuums in excess of 10^{-8} torr were routinely reached at temperatures in excess of 150°C.

For low temperatures, of -110 to -190°C, a 5 cubic foot vacuum chamber was modified so that the glass fiber filters containing the spores were in direct contact with a copper heat transfer system. Due to the low temperatures, outgassing was minimized and the final vacuum reached was lower, in the 10^{-10} torr region (Fig. 3).

Results and Discussion

The five test organisms showed marked differences in their ability to survive exposure to elevated temperatures while under ultrahigh vacuum (Table 1). Appreciable percentages of all of the test organisms were recovered after exposure to temperatures up to and including 60°C. Above this temperature only *B. subtilis* var. *niger* and *A. niger* were notably resistant to this combination of stresses. *A. niger* appeared to be the most resistant of the test organisms; an extremely small portion of the exposed spores were still viable after exposure to 107°C but not to 120°C. In comparison, no viable spores of *B. subtilis* var. niger were recovered after being heated to 100°C in an ultrahigh vacuum for 4 to 5 days. Exposure to -110 and -190°C and ultrahigh vacuum did not appear to be lethal to the test spores, which is not surprising since these conditions are somewhat comparable to the lyophilization technique so commonly employed for preserving microbial cultures.

Spores exposed to 90°C and atmospheric pressure presented a different survival pattern (Table 2). Of the five test organisms, only two, B. subtilis var. niger and much smaller numbers of B. megaterium, were recovered after 24 hours. B. subtilis var. niger was recoverable after incubation for 5 or more days at this temperature. A. niger, which was the most resistant test organism in ultrahigh vacuum, did not survive 24 hours exposure at 90°C.

With the exception of B. subtilis var. niger, ultrahigh vacuum appeared to protect B. stearothermophilus, B. megaterium, C. sporogenes and especially A. niger to prolonged exposure to elevated temperatures in the vicinity of 90°C. There appears to be a threshold temperature where this protective effect occurs since a comparison of recovery of these test spores at 60°C (Table 3) at atmospheric pressure and in ultrahigh vacuum indicates that at this temperature ultrahigh vacuum is not protective. Considering the large differences in resistivity between B. subtilis var. niger, A. niger and the remaining three test spores in ultrahigh vacuum it is conceivable that we are dealing with either an ability to prevent the vacuum distillation of essential cell constituents or a capability for maintaining sufficient cell integrity to enable a cell to resume growth upon rehydration. For some reason A. niger loses this property when dried at 90°C at atmospheric pressure.

Recovery data obtained by colony counts is often not indicative of what occurs at the individual cell level. In order to determine some aspects of the extent of damage to the spores after being heated in an ultrahigh vacuum, germination of B. subtilis var. niger was investigated by phase contrast microscopy. The results of a microscopic assay of B. subtilis var. niger for the percentage germinated, ungerminated and those spores capable of undergoing outgrowth are presented in Figs. 4 to 8 and in Table 4. It is seen that after exposure to temperatures up to 69°C for 5 days almost all of the spores either germinated or produced vegetative cells after 2 hours incubation at 37°C on a tryptone-glucose extract agar medium. Above 83°C outgrowth, even after 24 hours incubation ceased, and the percentage germination decreased with increasing temperatures until after exposure to 120°C, 87% of the spores remained refractile (Fig. 8).

Soils

Two types of soils, a garden soil obtained from the Cambridge vicinity and another from the Mohave desert were examined at various temperatures at ultrahigh vacuum. In Table 5 the garden soil showed quantitative recovery of mesophilic aerobes, mesophilic anaerobes, and actinomycetes, but no molds at temperatures up to 100°C. At higher temperatures a qualitative recovery procedure was employed, and mesophilic aerobes, molds and a large number of actinomycetes were recovered.

The higher recovery of anaerobes at 100°C over that at lower temperatures is the result of heat shocking the soil prior to plating - a treatment which has not influenced the recovery of C. sporogenes after vacuum-heat treatment.

One aspect of interest in this study is a comparison of the thermal resistivity of microorganisms exposed to elevated temperatures in ultrahigh vacuum to those exposed at atmospheric pressure. Of the test spores *A. niger* was extremely resistant at temperatures of 90°C and higher in an ultrahigh vacuum, but showed extreme susceptibility to 90°C at atmospheric pressure (Table 2). This indicated that a screening procedure for heat resistant organisms, employing an oven at atmospheric pressure, might not be capable of predicting whether certain organisms, which, due to a unique physiological response, might be capable of surviving drying at ultrahigh vacuums but not at atmospheric pressure. That this is indeed the case is shown by the following experiment.

Fifty isolates from desert soil exposed to 120°C and vacuum for 4.5 days were returned to the vacuum chamber on glass filters. Seven cultures were recovered which included six colorless, punctiform sporeformers and one amber, butyrous organism, as yet uncharacterized. Only one of these, a colorless punctiform bacillus colony type, survived 120°C for 3 hours at atmospheric pressure.

Protection by soil is indicated by the results shown in Table 6 for Mohave desert soil. Forty-three typical isolates which survived 170°C for 4-5 days at 6 x 10^{-9} torr were also screened at 120°C for 3 hours at atmospheric pressure. Only five bacteria survived this treatment. Future experiments will determine their resistivity in ultrahigh vacuum at elevated temperatures.

Bruch, Koesterer and Bruch (1962) had previously noted that mesophilic sporeformers in soil displayed a higher resistance to dry heat as compared to the isolates placed on filter paper strips.

Irradiation of Ultradried Organisms

Many organisms are more sensitive to ionizing radiation when they are irradiated in air than when they received the same radiation dose under anoxic conditions. This oxygen effect is known to occur in aqueous suspensions for a variety of organisms, and has been reported for X-irradiation dry spores of B. megaterium (Powers, 1961).

In our investigation, the gamma radiation resistance of extremely dry spores which had been in ultrahigh vacuum was compared with the resistance of similar spores which had been stored over silica gel. It was also possible to determine whether vacuum *per se* was lethal. Spores in ultrahigh vacuum were at pressures in the 10^{-9} mm Hg range. At these low pressures, the spores are extremely dry. Volatile external spore constituents as well as internal components which could pass the spore coat barrier were certainly being removed from the spores during the several days the spores were in vacuum.

Washed spores of *B. stearothermophilus*, *B. megaterium*, and *C. sporogenes* were dried on glass fiber filter circles at 45°C, and stored overnight over silica gel. About one million spores were pipetted onto each filter. The filters were mounted on a stainless steel wire support which was inserted into a glass tube fitted with an ultrahigh vacuum valve assembly and an ultrahigh vacuum gauge. It was possible to transport each assembly to the Cobalt-60 irradiator so that the tube contents were maintained under ultrahigh vacuum. A hot-filament ultrahigh vacuum gauge attached to each tube made it possible to determine pressures before and after the tubes were irradiated.

An ultrahigh vacuum assembly is shown in Fig. 9. Each assembly is about 21 inches in length and is attached to a vacuum chamber having a pumping arrangement identical to that shown in Fig. 1.

Spores were maintained in ultrahigh vacuum under continuous pumping for 4.5 - 5.5 days. The valves were then closed, and the 7 tubes were detached from the chamber for irradiation experiments and for plating as vacuum controls.

The tube-vacuum valve system is shown in detail in Fig. 10 with two tubes positioned in a rack ready for irradiation. Normally one tube was irradiated under vacuum and the other tube opened to admit dry air and then closed before irradiation.

Manifold System

Pressures in the individual tubes when loaded with six filters rose from 10^{-9} torr range before valve closure to about the middle 10^{-8} torr after valve closure. The entire sample assembly consisting of the ultra-high vacuum valve, the gauge and the sample tube were transported to a submerged pool type Co^{60} facility for gamma irradiation at an intensity of 5000 rad per minute.

In each experiment spores were irradiated while in vacuum, in dry air after vacuum treatment, and after storage over silica gel at atmospheric pressure. Filters were then blended, and appropriate diluations were plated on suitable media. Membrane filters were used where necessary. A portion of *B. stearothermophilus* blend was heated in a boiling water bath for 30 minutes before these spores were plated. With the exception of *B. stearothermophilus*, which was incubated at 55°C, all organisms were incubated at 30°C for 1 or 2 days as necessary for maximal counts.

The results are presented in Fig. 11 and Table 7. The radiosurvival curves shown in Fig. 11 indicate that *B. megaterium* is the most resistant and *C. sporogenes* the least resistant of the three organisms irradiated in the dry state. One question of interest is whether these dried spores are more or less radioresistant that those normally hydrated. The radioresistivity of the stock suspension of spores of *B. stearothermophilus* irradiated in phosphate buffer saturated with nitrogen was greater than for those spores irradiated in the dry state. A better comparison would be between dried spores and hydrated spores placed on glass filter discs and this data is now being collected. Nevertheless it is of pertinence that B. stearothermophilus spores were appreciably less radioresistant after ultradrying. Elimination of a portion of the indirect effects by removal of water was not of a sufficient magnitude to overcome what appears to be an inherently decreased radioresistivity. Other investigations indicate that bacterial spores may be less radioresistant in the dry state (Pepper, Buffa and Chandler, 1956), and Alper (1961) in her review indicated that moisture content may be a physiological factor in radioresistivity rather than a dose modifying factor such as air.

The presence of air does, in fact, modify the dried spores radioresistivity (Table 7). Column A is the percent survival of the organisms at various doses under vacuum and was illustrated in Fig. 11. The values in Columns B and C demonstrate that subsequent exposure to air of the spores dried in ultrahigh vacuum increased their sensitivity to gamma radiation. Moderate drying over a desiccant and subsequent irradiation in air (Column D) resulted in a radioresistivity greater than that noted for the ultradry spores (Column B) also exposed to air and of equivalent survival to that noted for ultradried spores irradiated in vacuum (Column A).

Tallentire (1958) noted that spores of B. subtilis dried on kaolin under low vacuum (10^{-3} torr) for 6 hours experienced an oxygen effect when irradiated with gamma rays. He stated that oxygen contributes to direct rather than to indirect radiation effects on the spore. The data in Table 7, obtained at a much higher vacuum for considerably drier spores, appears to substaniate the observation of Tallentire and also that of Powers (1961) for the oxygen dependent damage to spores of B. megaterium.

Undoubtedly the interrelationships between moisture content, its effect on the physiological organization of the spore and the composition of the gaseous phase are complex and require further clarification.

These experiments are continuing, and it is intended to compare the radioresistivities of microorganisms in various hydrated states at different oxygen levels in addition to combining heat with irradiation on ultradried spores.

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TABLE 1

Per Cent Survival of Spores Following Exposure

To Ultrahigh Vacuum at Various Temperatures for Five Days

Organism	-190°C	-110°C	25°C	53°C	60°C	88°C	100°C	107°C	120°C
_B_. _stearothermophilus_	118	67	67	14	1.2	0.001	-	-	-
_B_. _subtilis_ var. _niger_	93	119	113	63	40	0.01	NG*	NG*	-
_B_. _megaterium_	88	90	98	2.6	0.7	<0.001	-	-	-
_C_. _sporogenes_	125	83	88	<1	0.1	<0.01	-	-	-
_A_. _niger_	126	83	98	-	25	0.2	0.03	G**	NG*

* No Growth (NG): $< 2 \times 10^{-6}$ per cent

** Growth (G): $< 6 \times 10^{-3}$ per cent

TABLE 2

Survival of Spores at 90°C and Atmospheric Pressure

Organism	1 day	2 days	3 days	4 days	5 days	6 days	7 days
B. stearothermophilus	0	-	-	-	-	-	-
B. subtilis var. *niger* (Expt. A)	48	26.5	11	3.1	0.55	0.08	0.002
B. subtilis var. *niger* (Expt. B)	32.6	12.6	4.2	0.46	0.09	-	-
B. megaterium	0.005	0	-	-	-	-	-
C. sporogenes	0	-	-	-	-	-	-
A. niger	0	-	-	-	-	-	-

TABLE 3

Per Cent Survival of Dry Spores During Five Days at 25°C and 60°C in Vacuum and at Atmospheric Pressure

	Atmospheric Pressure				Ultrahigh Vacuum		
	Days at 60°C			Days at 25°C	Days at 60°C		Days at 25°C
	0	4	5	5	0	4-5	4-5
Organism	spores/filter	%	%	%	spores/filter	%	%
B. stearothermophilus	1.6×10^6	30	35	56	1.3×10^5	1.2	67
B. subtilis var. niger	1.8×10^6	67	72	78	1.8×10^5	40	113
B. megaterium	1.5×10^6	80	87	87	1.8×10^5	0.7	98
C. sporogenes	1.6×10^6	48	62	106	3.0×10^5	0.1	88
A. niger	0.7×10^6	61	9	120	1.3×10^5	25	98

TABLE 4

Germination of B. subtilis var. niger Spores and Refractility Following Exposure to Ultrahigh Vacuum at Various Temperatures for Five Days

Vacuum Chamber Temperature-°C	TGE Agar			1.5% Agar	
	Non-refractile %	Refractile %	Outgrowth %	Non-refractile %	Refractile %
25°Control	11	1	88	9	91
20°	21	3	77	6	94
40°	23	2	75	15	85
52°	58	1	41	13	87
69°	85	0	15	12	88
83°	98	2	< 0.1	17	83
90°	92	8	< 0.1	-	-
100°	89	11	0	5	95
107°	64	36	0	5	95
120°	13	87	0	-	-

TABLE 5

Per Cent Survival of Organisms in Garden Soil

Exposed to Ultrahigh Vacuum at Various Temperatures for Five Days

	40°C	52°C	69°C	90°C	100°C	110°C	120°C	170°C
Mesophilic Aerobes	30.4	15.4	7.8	0.15	0.3	0.03	0	+
Mesophilic Anaerobes	1.3	<0.002	<0.002	<0.002	0.06	0.02	0	0
Molds	100	100	60	2.0	-	0	0	+
Actinomycetes	60.5	38	25.8	14.2	12.6	0.8	+[1]	+

1 - Qualitative - Growth occured in recovery broth from one gram of soil

TABLE 6

Microbiological Analysis of Mohave Desert Soil

Exposed to 170°C for 4.5 Days at 6×10^{-9} torr

	Organisms per gram		
	Bacteria	Molds	Actinomycetes
Mesophilic Aerobes	22	14	4
Thermophilic Aerobes	1	0	0
Mesophilic Anaerobes	9	-	0
Thermophilic Anaerobes	5	-	0

TABLE 7

Per Cent Survival of Spores Irradiated in Vacuum
and in Air After Being Dried in Ultrahigh Vacuum

	Dose (rad x 10^3)	A $\frac{IV}{UV}(100)$	B $\frac{IVO}{UV}(100)$	C $\frac{IVO}{IV}(100)$	D $\frac{IA}{UA}(100)$
B. stearothermophilus	100	15	9	59	30
	200	12	1	8.5	6.5
	300	1.3	0.01	0.41	1.8
	500	0.3	*	*	*
B. megaterium	100	86	53	61	61
	200	43	8.3	20	27
	300	9.3	0.47	5	2.7
	400	1.5	0.06	4	1.7
C. sporogenes	100	20	14	74	51
	200	17	0.3	2	19
	300	1.4	*	*	7
	400	0.14	*	*	0.12

I - Irradiated U - Unirradiated O - Opened to Air
V - Vacuum A - Air, stored in desiccator
* - No detectable survivors at this irradiation dose

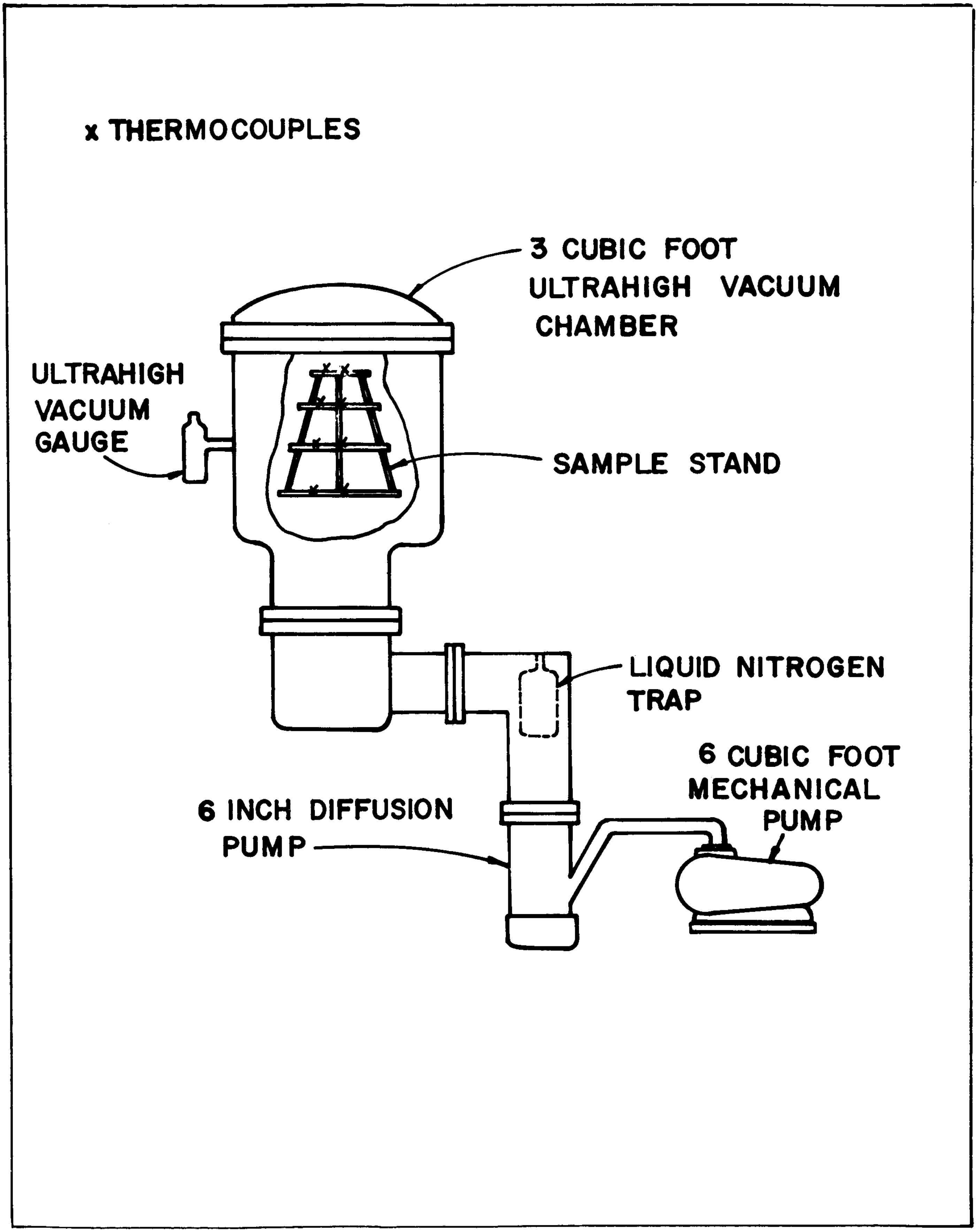


FIGURE 1

ULTRAHIGH VACUUM SYSTEM FOR AMBIENT AND

ELEVATED TEMPERATURE EXPERIMENTS

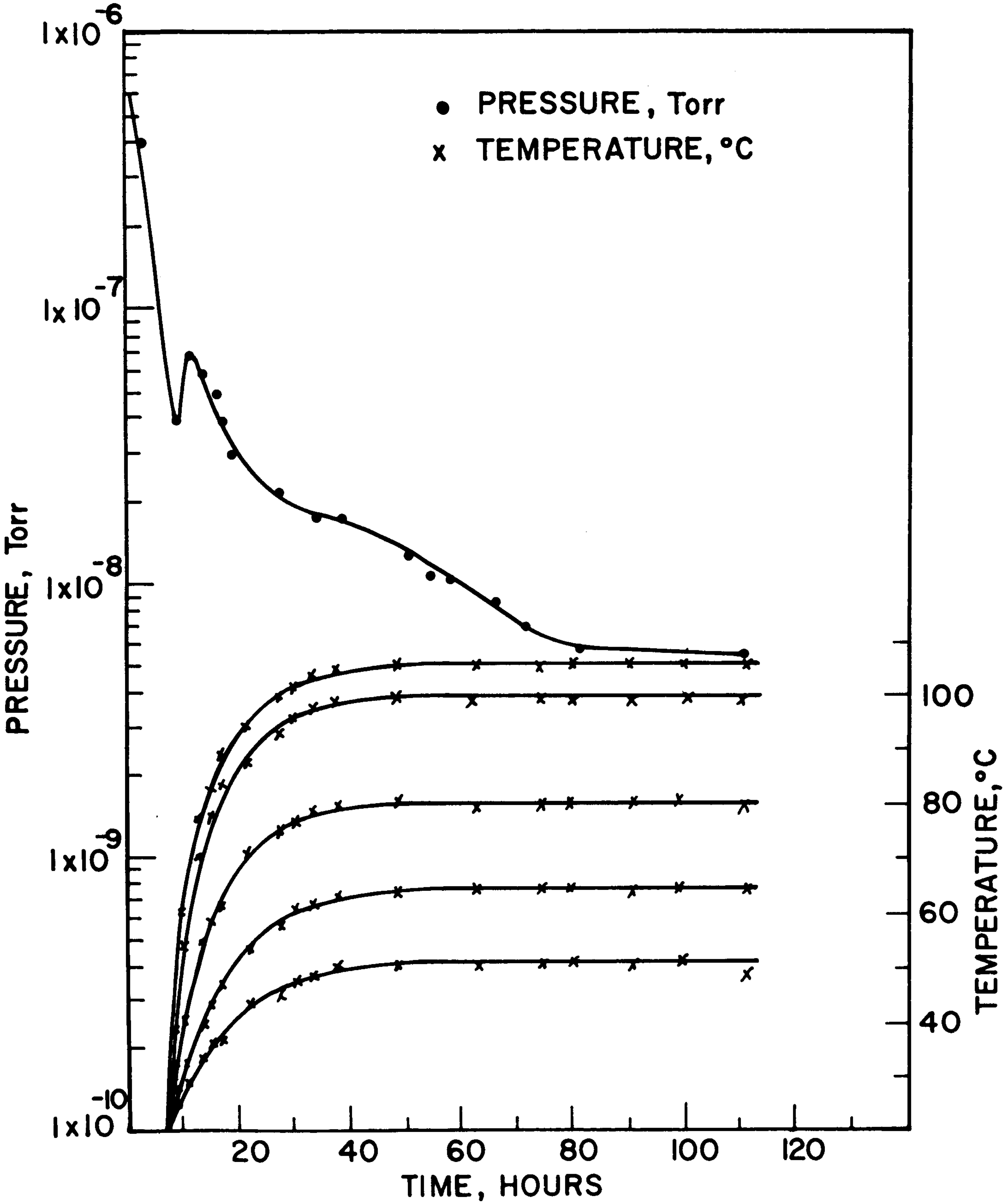


FIGURE 2

THE VERTICAL TEMPERATURE GRADIENT AT FIVE LEVELS

IN AN ULTRAHIGH VACUUM SYSTEM

HEATED BY AN EXTERNAL RESISTANCE HEATER

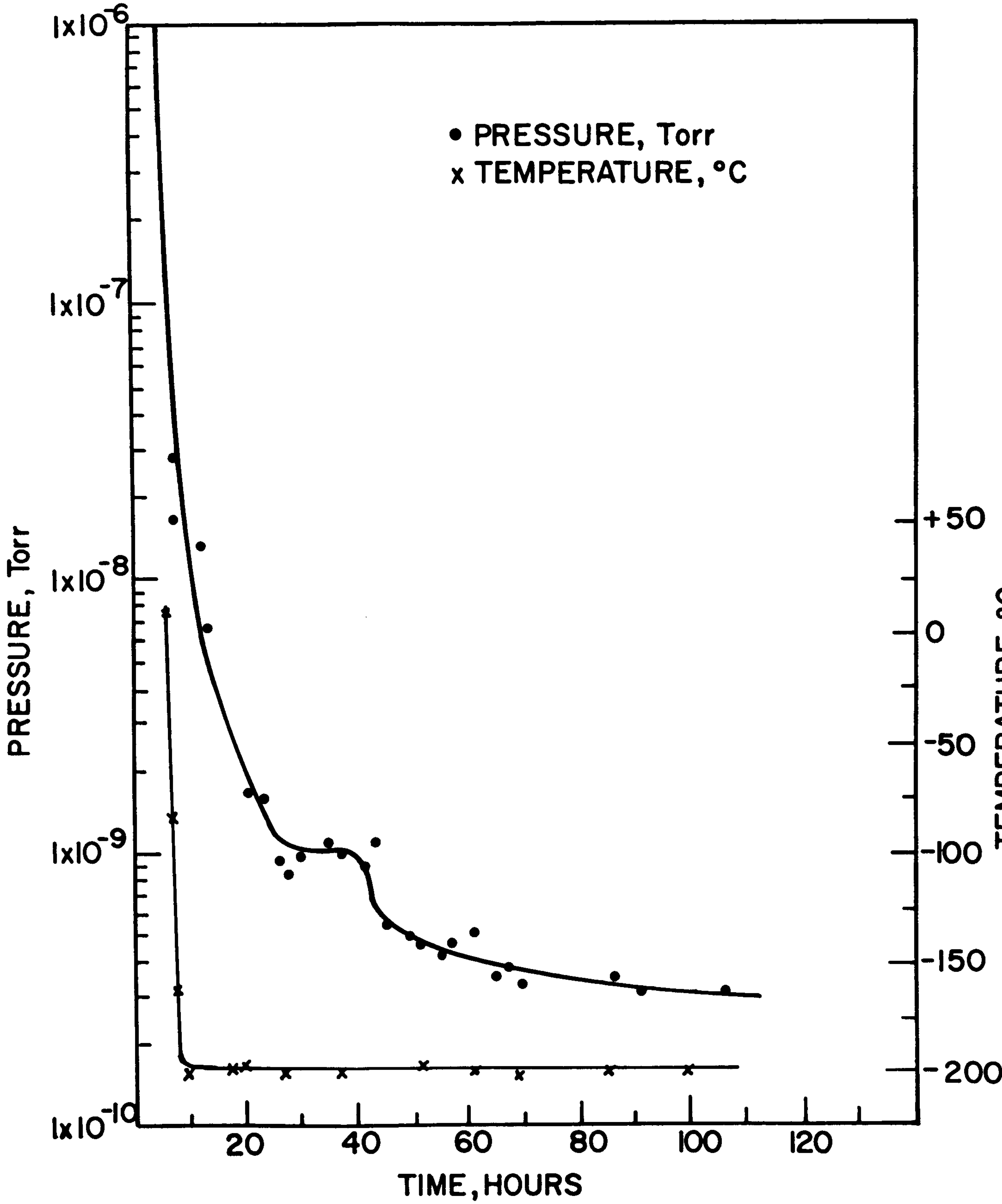
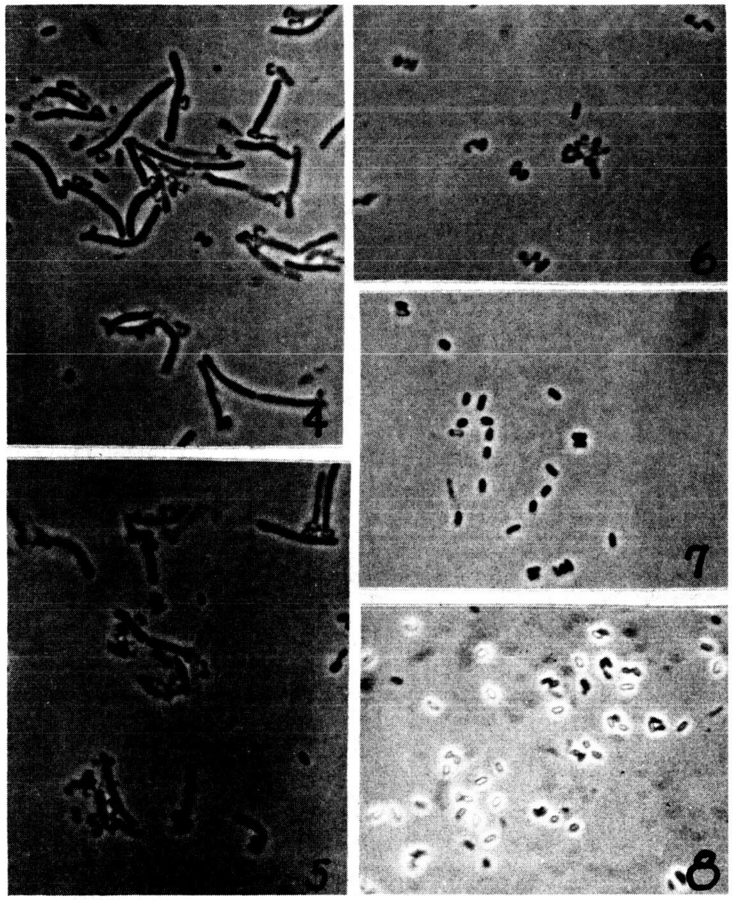


FIGURE 3

PRESSURE-TEMPERATURE-TIME RELATIONSHIPS IN AN ULTRAHIGH VACUUM SYSTEM COOLED WITH LIQUID NITROGEN



FIGURES 4 - 8

B. SUBTILIS VAR. NIGER SPORES EXPOSED TO ULTRAHIGH VACUUM AT VARIOUS TEMPERATURES AND SUBSEQUENTLY INCUBATED AT 37°C ON TRYPTONE GLUCOSE EXTRACT AGAR

Figure 4 - Control not exposed to vacuum; 2 hour incubation

Figure 5 - 40°C; 2 hour incubation

Figure 6 - 69°C; 2 hour incubation

Figure 7 - 83°C; 24 hour incubation

Figure 8 - 120°C; 24 hour incubation

FIGURE 9

A COMPLETED ULTRAHIGH VACUUM ASSEMBLY CONNECTED TO THE MAIN VACUUM CHAMBER USED

FOR GAMMA RADIATION OF TEST SPORES

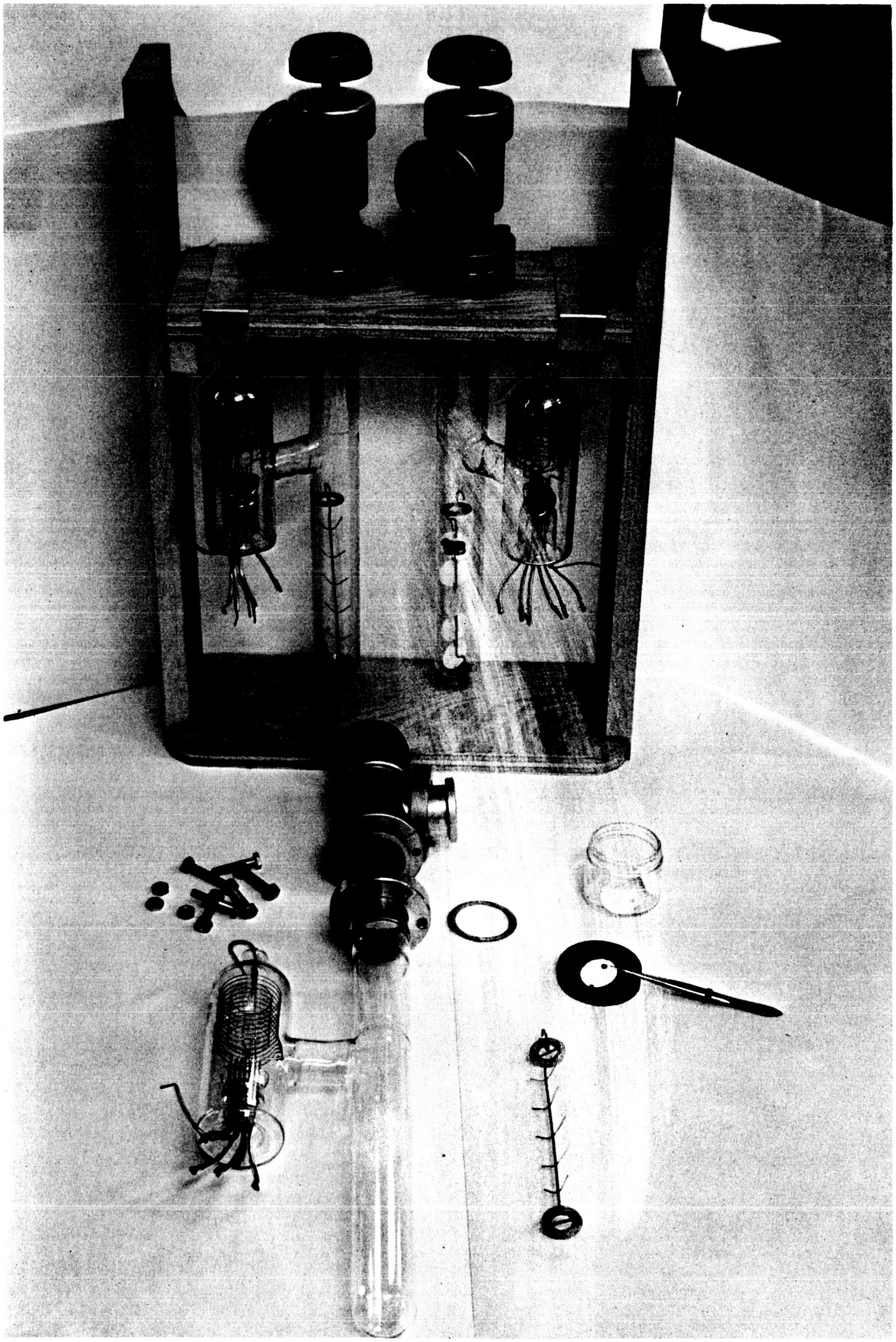


FIGURE 10

THE COMPONENT PARTS OF AN ULTRAHIGH VACUUM ASSEMBLY AND THE FRAME USED FOR EXPOSING THE ASSEMBLY TO GAMMA RADIATION

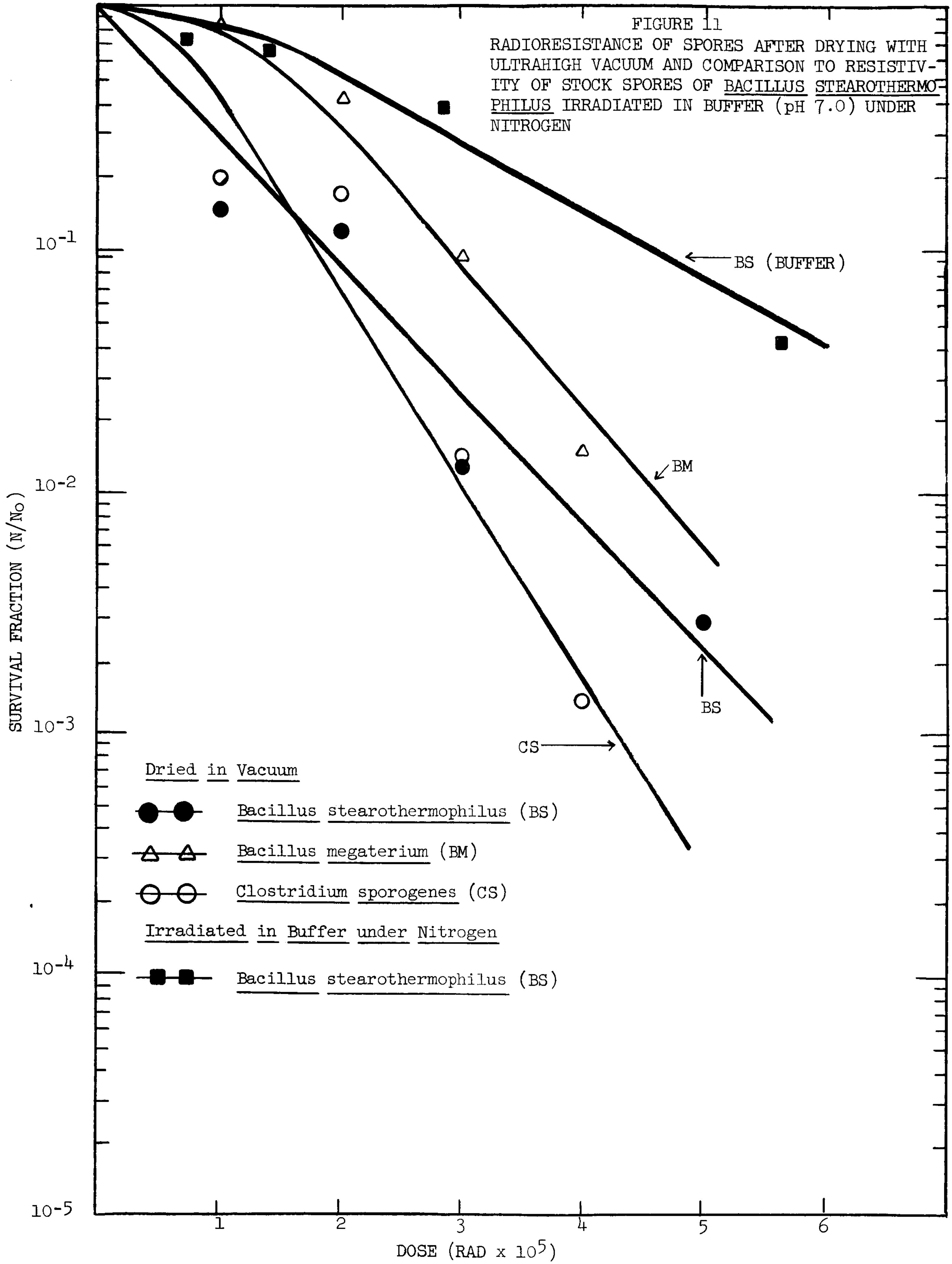


FIGURE 11
RADIORESISTANCE OF SPORES AFTER DRYING WITH ULTRAHIGH VACUUM AND COMPARISON TO RESISTIVITY OF STOCK SPORES OF BACILLUS STEAROTHERMOPHILUS IRRADIATED IN BUFFER (pH 7.0) UNDER NITROGEN
BS (BUFFER)
BM
BS
CS
Dried in Vacuum
Bacillus stearothermophilus (BS)
Bacillus megaterium (BM)
Clostridium sporogenes (CS)
Irradiated in Buffer under Nitrogen
Bacillus stearothermophilus (BS)
SURVIVAL FRACTION (N/N_0)
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
1
2
3
4
5
6
DOSE (RAD x 10^5)